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Immunohistochemical Expression of Cytokeratins in Intrahepatic Cholangiocarcinoma and Metastatic Adenocarcinoma of the Liver

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Background and Objectives: This study was designed to identify a difference in immunostaining that might help to distinguish between primary and metastatic liver neoplasms.
Methods: We examined immunohistochemical expression of cytokeratins (CKs) 7, 8, 19, and 20 in 12 intrahepatic cholangiocarcinomas (ICCs; 9 of the mass-forming and 3 of the infiltrating type), 25 metastatic colorectal carcinomas (MCCs), and 7 metastatic gastric carcinomas (MGCs) of the liver.
Results: CKs 7 and 19 were expressed in all ICCs of infiltrating type, while each was seen in 7/9 (77.8%) of mass-forming type. CK 7-positive/CK 20-negative was seen in 9/12 (75.0%) of ICCs and in none of the 25 MCCs, while CK 7-negative/CK 20-positive was seen in 1/12 (8.3%) of ICCs and 20/25 (80.0%) of MCCs. No differences were observed between MGCs and ICCs.
Conclusions: These results suggest that immunohistochemical staining for both CKs 7 and 20 is useful for the differential diagnosis of ICCs and MCCs, whereas phenotypic expression of CKs appears to be different between mass-forming and infiltrating types of ICCs.
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KEY WORDS: **cytokeratin; intrahepatic cholangiocarcinoma; metastatic carcinoma; colorectal carcinoma; liver; immunohistochemistry**

INTRODUCTION

With progress in diagnostic and surgical procedures and in postoperative management, hepatic resection has become an increasingly frequent treatment for both primary and metastatic carcinomas of the liver. It is very important that the hepatic tumor be correctly diagnosed as a primary or a metastatic carcinoma because the postoperative treatment of the patients and the outcome differ between those patients with intrahepatic cholangiocarcinomas (ICCs) and those with metastatic carcinomas.

Among primary epithelial malignant neoplasms of the liver, hepatocellular carcinoma (HCC) is the major histological type, followed by ICC [1]. Many immunohistochemical studies aimed at the differential diagnosis of HCC and ICC have been reported [2–8]. However, it is difficult to distinguish histologically between metastatic adenocarcinoma and ICC because both tumors have a histological configuration similar to that of adenocarcinoma.

Many normal and neoplastic epithelial cells express cytokeratins (CKs). However, expression of CKs differs from differentiation of the epithelial cells [9]. The phenotypic expression of CKs has been studied in neoplastic and nonneoplastic cells in the liver. Ordinary HCC cells express CK 8 and CK 18, while ICC cells also express

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CK 7 and CK 19 [9]. Therefore, detection of the expression of CK 7 and CK 19 has been proposed as a possible method for establishing the cellular origin of primary liver carcinomas [2,4,6,7,9]. CK 20, which was recently detected as a new CK polypeptide, is a cytoplasmic intermediate-filament protein that was first isolated from villi of the duodenal mucosa [10]. Antibodies against CK 20 react with the gastrointestinal mucosa and with carcinomas arising from the stomach and colorectum. In the liver, hepatocytes do not react with such antibodies, and a very few epithelial cells in the intrahepatic bile ducts react weakly with the antibodies [11].

Some investigators have classified ICC according to macroscopic appearance [12–14]. One group of investigators classified tumors as either mass-forming or infiltrating and demonstrated that the mode of spreading of ICC differs depending on the macroscopic appearance: ICC of the mass-forming type spreads in the liver through the portal blood flow, with formation, frequently, of multiple metastatic lesions in the liver [12]. Formation of such multiple lesions by the spread of ICC of the mass-forming type makes it difficult to diagnose the tumors both clinically and pathologically. On the other hand, metastatic liver carcinomas originated from colorectal cancer sometimes show intrabiliary growth and cause obstructive jaundice, and such growth pattern is easily confused with ICC of the infiltrating type [15].

Recently, one group of investigators demonstrated that expression of immunohistochemical CKs are useful in the differential diagnosis of primary liver carcinomas and metastatic colorectal carcinomas (MCCs) of the liver [16]. However, in their study, ICCs were not classified into the subtypes and only MCCs were mentioned.

In this report, we describe the results of a comparative immunohistochemical study of 12 ICCs (9 of mass-forming and 3 of infiltrating type) and 32 metastatic adenocarcinomas of the liver [7 metastatic gastric carcinomas (MGCs) and 25 MCCs]. This study was designed to identify a difference in immunostaining that might help to distinguish between these neoplasms.

MATERIALS AND METHODS

Surgically removed tumor tissues from 12 cases of ICCs and 32 cases of metastatic adenocarcinomas of the liver were investigated by immunohistochemical staining. Eleven of the ICCs and all the metastatic adenocarcinomas had been resected at the Department of Surgery I, Oita Medical University, from 1988 to 1996, and one ICC at Oita National Hospital in 1996. All but one of the tumors had been diagnosed histologically as adenocarcinoma, and the remaining one had been diagnosed as adenocarcinoma with focally squamous metaplasia of the liver.

ICC was defined as a tumor arising from a segmental duct or from a more peripheral duct. The hilar-type ICC was excluded from the study. ICC was classified according to macroscopic appearance: nine tumors were of the mass-forming type and three were of the infiltrating type. A mass-forming tumor was defined as a nodular tumor with a relatively clear margin; an infiltrating tumor was defined as a nonnodular tumor that extended mainly along the intrahepatic bile duct. There were no tumors with predominantly papillary growth into the lumen of the bile duct. We also examined 25 MCCs and 7 MGCs.

Each surgical specimen was fixed in 10% formalin and processed routinely for light microscopy. Paraffin-embedded blocks were sectioned and the sections were stained with hematoxylin and eosin. Selected specimens were studied immunohistochemically by the avidin-biotin complex method. The antibodies used were mouse monoclonal antibodies against CK 7 (DAKO, Carpinteria, CA; diluted 1:50), CK 8 (DAKO; diluted 1:50), CK 19 (DAKO; diluted 1:50), and CK 20 (DAKO; diluted 1:50). The sections were predigested with 0.1% protease (Sigma Chemical, St. Louis, MO) in phosphate-buffered saline at 37°C for 5 min to unmask binding sites before immunoperoxidase reactions for all primary antibodies. When more than 10% of neoplastic cells in a sample gave a positive immunoreaction with a specific antibody, the tumor was defined as positive for the corresponding antigen.

RESULTS

Pathologic Findings

Intrahepatic cholangiocarcinomas were classified into two groups according to their macroscopic appearance: mass-forming and infiltrating. Histologically, most ICCs of the mass-forming type showed configuration of poorly differentiated adenocarcinoma: as a solid or microglandular structure with scanty fibrous stroma (Fig. 1A). In addition, eight of nine ICC tumors of the mass-forming type had invaded the peripheral portal vein and/or formed a number of metastatic lesions around the tumor (Fig. 2). By contrast, tumors of the infiltrating type were composed of well-differentiated adenocarcinoma: macroglandular or papillary structure with abundant fibrous stroma (Fig. 1B). Only one ICC of the infiltrating type had some features of squamous metaplasia. Various degrees of tumor necrosis were observed in cases of ICC of both types but no extensive necrosis was apparent in the central area of each tumor.

All the metastatic carcinomas showed the configuration of well to moderately differentiated adenocarcinoma, being glandular structures of various sizes with abundant fibrous tissue. Extensive necrosis was frequently observed in the central area of the tumor. In some cases of the MCCs, invasion of the peripheral portal vein was observed around the tumor, similar to the invasion pattern seen with ICCs of the mass-forming type (Fig. 3).

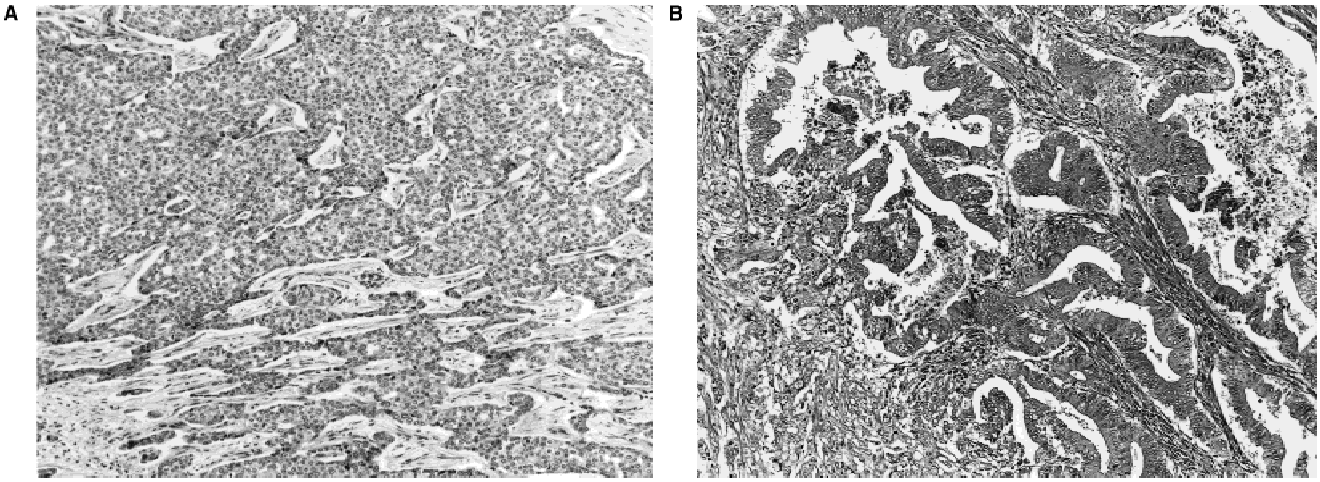

Fig. 1. **(A)** Poorly differentiated intrahepatic cholangiocarcinoma (ICC) of mass-forming type shows solid or microglandular structure with scanty fibrous stroma. **(B)** Well-differentiated ICC of infiltrating type shows macroglandular or papillary structure with abundant fibrous stroma (hematoxylin-eosin stain).

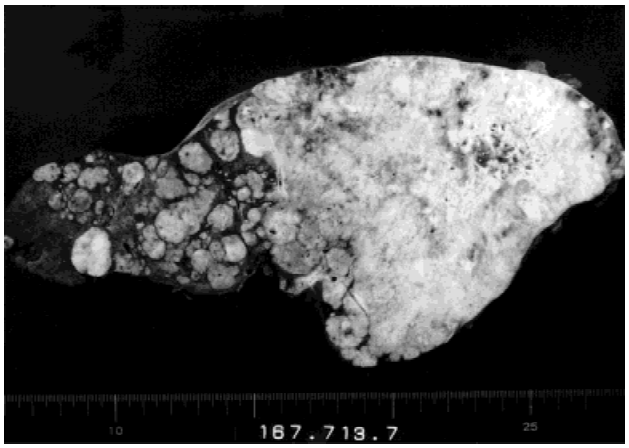

Fig. 2. Macroscopic appearance of ICC of mass-forming type. Small multiple lesions are found around the tumor in the liver.

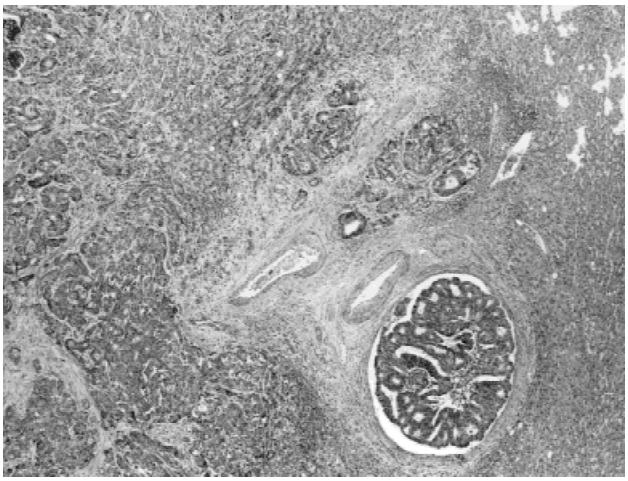

Fig. 3. Microscopic view of a metastatic colorectal carcinoma (MCC) of the liver. The neoplastic cells are configured of well to moderately differentiated adenocarcinoma. Invasion to the peripheral portal vein around the tumor is noted (hematoxylin-eosin stain).

Immunohistochemical Staining of Cytokeratins

The results of immunohistochemical staining of CKs are summarized in Table I. Seven of nine (77.8%) ICCs of the mass-forming type were regarded as positive for CK 7; one of the two tumors regarded as negative reacted only focally and the other showed no reaction. Similarly, eight of nine (88.9%) ICCs of the mass-forming type were regarded as positive for CK 8 and the remaining one tumor reacted only focally. All ICC tumors were positive for CK 19 to various extents, but the number of neoplastic cells in two ICCs of the mass-forming type was less than 10% of all neoplastic cells. Moreover, the number of immunopositive neoplastic cells and the intensity of immunostaining for CK 19 in ICCs of the mass-forming type tended to be lower than those in ICCs of the infiltrating type (Figs. 4A and 4B). All except two ICCs were regarded as negative for CK 20; one ICC of the mass-forming type and one of the infiltrating type were positive for CK 20 with diffuse and strong immunostaining.

All except one of the MCCs were regarded as negative for CK 7. The one CK 7-positive sample was immunostained diffusely and strongly. All MCCs were positive for CK 8. Ten of 25 MCCs were regarded as negative for CK 19. Of these, seven were completely negative and three were immunostained only focally. Twenty-one of 25 (84.0%) MCCs were regarded as positive for CK 20 (Fig. 4C), and only one of the four defined as negative tumors was completely negative for the antibody. By contrast, the positive expression rates of CKs in MGCs were similar to that in ICC of the mass-forming type.

The expression pattern of CK 7 and CK 20 in ICCs and metastatic carcinomas is summarized in Table II. The staining pattern of CK 7-negative/CK 20-positive was

TABLE I. Results of Immunohistochemical Staining of Intrahepatic Cholangiocarcinomas and Metastatic Adenocarcinoma*

	Number of cases	CK 7$^+$ (%)	CK 8$^+$ (%)	CK 19$^+$ (%)	CK 20$^+$ (%)
ICC	12 (100)	10 (83.3)	11 (91.7)	10 (83.3)	2 (16.7)
Mass-forming type	9 (100)	7 (77.8)	8 (88.9)	7 (77.8)	1 (11.1)
Infiltrating type	3 (100)	3 (100)	3 (100)	3 (100)	1 (33.3)
Metastatic carcinoma	32 (100)	4 (12.5)	31 (96.9)	21 (65.6)	22 (68.8)
Stomach	7 (100)	3 (42.9)	6 (85.7)	6 (85.7)	1 (14.3)
Colorectum	25 (100)	1 (4.0)	25 (100)	15 (60.0)	21 (84.0)

*ICC, intrahepatic cholangiocarcinoma; CK, cytokeratin; +, more than 10% of the neoplastic cells gave a positive immunoreaction; ( ), frequency of immunopositive cases with the indicated profile.

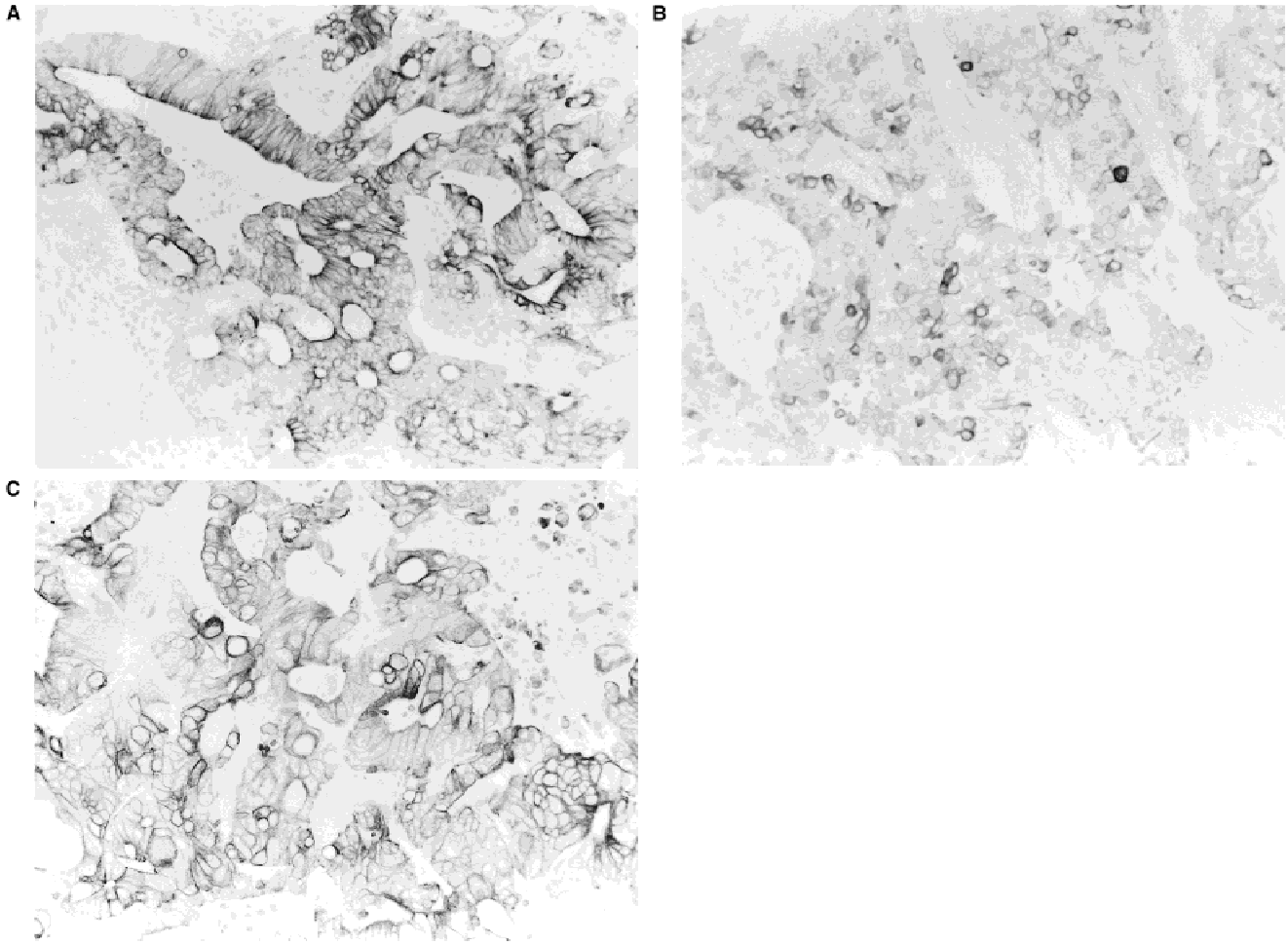

Fig. 4. **(A)** ICC of infiltrating type with strongly positive reaction for cytokeratin (CK) 19. **(B)** ICC of mass-forming type with weaker positive reaction for CK 19. **(C)** MCC with strongly positive reaction for CK 20 (A–C: immunoperoxidase).

seen in 20 of 25 (80.0%) of MCCs but in only 1 of 12 (8.3%) ICCs. By contrast, the staining pattern of CK 7-positive/CK 20-negative was seen in 9 of 12 (75.0%) of ICCs, but in none of the MCCs. There were only a few tumors with atypical expression of CK 7 and CK 20. One MCC was CK 7-positive/CK 20-positive and one ICC of the mass-forming type was CK 7-negative/CK 20-positive. In addition, one ICC of the mass-forming type and four MCCs were CK 7-negative/CK 20-negative.

DISCUSSION

Most malignant neoplasms of the liver are metastatic deposits. Nizze et al. [17] evaluated primary and secondary malignant liver tumors obtained as autopsy, biopsy, and cytological specimens and reported that the ratio of

TABLE II. Detection of the Expression of Cytokeratins 7 and 20 in ICCs and Metastatic Carcinomas of the Liver*

	Number of cases	CK 7^-/CK 20^-	CK 7^-/CK 20^+	CK 7^+/CK 20^-	CK 7^+/CK 20^+
ICC	12 (100)	1 (8.3)	1 (8.3)	9 (75.0)	1 (8.3)
Mass-forming type	9 (100)	1 (11.1)	1 (11.1)	7 (77.8)	0 (0)
Infiltrating type	3 (100)	0 (0)	0 (0)	2 (66.7)	1 (33.3)
Metastatic carcinoma	32 (100)	7 (21.9)	21 (65.6)	3 (9.4)	1 (3.1)
Stomach	7 (100)	3 (42.9)	1 (14.3)	3 (42.9)	0 (0)
Colorectum	25 (100)	4 (16.0)	20 (80.0)	0 (0)	1 (4.0)

*( ), values are percentages of tumors that expressed the indicated combination of antigens.

primary to secondary liver tumors was 1:10 at autopsy, 1:6 in the biopsy series, and 1:3 in the cytological samples. Moreover, the most frequent primary cancers registered at biopsy were tumors of the colorectal, biliary, pancreatic, and gastric tissues. Thus, pathologists frequently encounter patients with hepatic tumors that are malignant deposits of tumors from other organs and/or with a history of such primary tumors in routine pathological examinations. Under such conditions, it is often difficult to determine whether the hepatic tumor is a primary or a metastatic carcinoma, especially when the hepatic tumor is present in isolation. It is clearly very important for pathologists to be able to distinguish a metastatic carcinoma from a primary carcinoma.

CKs are intermediate-filament proteins, as are vimentin, desmin, neurofilament, and glial filament. CKs are known to be classified into at least 20 types with molecular masses raging from 40,000 to 68,000 Daltons [9–11]. In these, CK 20 was identified by Moll et al. [10] in 1990 as protein IT of the intestinal cytoskeleton, which had been isolated from villi of the duodenal mucosa. They investigated immunohistochemically the distribution of CK 20 in human tumors and found that CK 20 was confined to the gastric and intestinal epithelia, urothelia, and Merkel cells. In their study, the expression of CK 20 in each carcinoma resembled that seen in the corresponding normal epithelium at the site of origin of the tumor. CK 20 was detected in the vast majority of adenocarcinomas of the colon (95.6%), mucinous ovarian tumors, transitional cell, and Merkel cell carcinomas and frequently also in adenocarcinomas of the stomach, bile system, and pancreas. Most squamous cell carcinomas and adenocarcinomas from other sites (breast, lung, endometrium), nonmucinous tumors of the ovary, and small-cell lung carcinomas were essentially or completely negative for CK 20. Sixteen (84.2%) of 19 adenocarcinomas of the gallbladder and bile ducts were positive for CK 20 to some degree and only 3 of 19 (15.8%) were completely negative [10,11]. In the present study, all but two ICCs were completely negative for CK 20. This disparity of immunohistochemical results suggests that the differentiation or cell origin might differ between intrahepatic and extrahepatic cholangiocarcinoma.

Many investigators have attempted to distinguish HCC from ICC or metastatic adenocarcinoma by monitoring the expression of CKs and other markers [2–8,18,19]. Hulimann et al. [7] demonstrated that phenotypes suggestive of HCC included expression of CK 8, CK 18, factor XIIIa, alpha-fetoprotein (AFP), C-reactive protein, and carcinoembryonic antigen (CEA) cross-reacting antigen. On the other hand, the expression of the following antigens effectively excluded HCC: CK 1, CK 5, CK 10, CK 11, CK 19, true CEA, and C-reactive protein. Christensen et al. [5] demonstrated that canalicular staining with polyclonal antibodies against CEA was specific for HCC, while negative results with monoclonal antibodies against CEA and against keratin (AE1/AE3) were suggestive of hepatocellular differentiation. Fisher et al. [2] demonstrated that, since metastatic carcinomas and carcinoid tumors of the large intestine were positive for CK 18 and CK 19 but not for CK 7, these tumors could be distinguished from primary and other metastatic tumors of the liver. Moreover, Maeda et al. [16] demonstrated the utility of CK 7 and CK 20 in differential diagnosis of ICCs and MCC of the liver. In the present study, although we found some exceptions, most cases of ICC and MCC could be differentiated by examining the expression of CK 7 and CK 20. The CK 7-negative/CK 20-positive pattern was seen in one ICC of the mass-forming type, whereas the CK 7-positive/CK 20-negative pattern was not seen in any MCCs. Therefore, the combination of CK 7 and CK 20 was more useful for distinguishing MCC from ICC than CK 7 alone. In other organs (lung and ovary), detection of the expression of CK 7 and CK 20 has been reported to be useful for differential diagnosis of primary and metastatic carcinoma [20,21].

Yamamoto et al. [12] classified ICCs as mass-forming or infiltrating. They demonstrated that the mass-forming type tended to generate intrahepatic metastasis, in particular near the main lesion, while the infiltrating type spread via Glisson's capsule and metastasize to hilar

lymph nodes. D'Errico et al. [22] studied primary liver carcinomas to assess the utility of the CK profile and detection mRNA for albumin, and demonstrated that peripheral ICC had a different phenotype from hilar and large duct cholangiocarcinomas. Moreover, the CK profile and the level of expression of mRNA for albumin in peripheral ICC showed many similarities to those of some HCCs. In our study, poorer differentiation and less intense immunostaining of CK of the bile duct type were frequently seen in ICCs of the mass-forming type as compared to ICCs of the infiltrating type. These observations suggest that the ICC of the mass-forming type resembles HCC and is different from the ICC of the infiltrating type.

The immunohistochemical detection of CK 7 and CK 20 seems to provide a helpful method for distinguishing ICCs from MCCs of the liver and phenotypic expression of CKs appears to differ between the mass-forming and infiltrating-type ICCs. In addition, it is still difficult to distinguish MGCs from ICCs by the current method of CK immunostaining.

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